

Hans Grundsell

**SOME EFFECTS OF COPPER AND "INERT" IUDS ON THE
ENDOMETRIUM. AN EXPERIMENTAL STUDY DURING
THE PREIMPLANTATIONAL PERIOD IN RABBITS.**

Lund 1975



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BY

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INTRODUCTION

Intrauterine devices (IUDs) for fertility control have been used at various times for more than 2000 years (135,136). In 1909, Richter (125) and later in 1928 Gräfenberg (52) described different types of IUDs. However, this type of contraception was not generally accepted until Oppenheimer (115) and Ishihama (68) in 1959 made their reports. During the following decade, IUDs consisting of various synthetic plastics, often called "inert" IUDs, were extensively used and studied. This type of IUD has a Pearl index of approximately 2-6 (i.e. 2-6 pregnancies in 100 woman-years). A new era in the history of IUDs began when Doyle and Clewe (37) and Zipper (158) in 1968-69 created "active" IUDs by adding progestins and metallic copper to "inert" IUDs, thereby increasing the antifertility effect of "inert" devices. Thus the addition of metallic copper to Tatum's T (a small plastic device) reduced the Pearl index from 18 to about 2 (143,144,158).

Despite considerable research into the mode of action of IUDs in experimental animals and in humans, their mechanism of action is still obscure. The effect of IUDs has been studied in several species and an antifertility effect is always obtained. Different stages of the reproductive process are reported to be affected by their presence, e.g. sperm transport, ova transport, corpus luteum function, blastocyst development and endometrial function (1,11,12,17,18,19,29,41,42,45,46,69,73,94,103,104,108,109,122,123,128,146,148,155; for further details see reviews cited below). So far, it has proved impossible to explain the mechanism of action of IUDs in a manner that applies to all studied species. The described differences in action are possibly due to species differences and probably also to the marked variation in size, configuration and composition of the devices. Only rarely, have different effects of the same type of device been studied in the same species in the same experimental model.

Several comprehensive reviews of the subject are published (26,33,36,40,41,43,54,55,75,85,105,131,143,144). Recently, a literature survey on copper IUDs in humans was published (116).

Effect of "inert" IUDs on the endometrium

Traumatic effects, e.g. local pressure atrophy, superficial erosions and necroses were generally found in the endometrium of both women (9,14,59,60,63,70,113,127,129,149) and animals (3,15,28,84,91,102). Normal (60,70,139), premature (153,154,155) and delayed (5,9,129,149) development of the endometrium has been reported. In rats, there is an inhibition of the decidual reaction (28,35,67,78,101). Thus, interference by "inert" IUDs with the normal development of the endometrium might be partly responsible for the antifertility effect of IUDs.

Several authors, e.g. Segal 1971 (131), have pointed to the importance of the inflammatory reaction for the antifertility effect of IUDs. Greenwald (51) found implantations only when the IUD failed to elicit an inflammatory response in rat endometrium. Similar results have been obtained in several species (47,48,61,74,103,106). Parr et al. (118) and El Sahwi and Moyer (43) found a relation between the number of leukocytes in the uterine fluid and the antifertility effect of IUDs.

Various endometrial and uterine enzymes are thought to be of importance for endometrial function and for implantation. Among the most frequently studied enzymes in the presence of "inert" IUDs are acid- and alkaline phosphatase and beta-glucuronidase. The activity of alkaline phosphatase was either uninfluenced or increased in various species (20,60,63,81,83,91,132). Acid phosphatase and beta-glucuronidase activities are generally reported to be increased in the presence of "inert" IUDs (11,20,80,81,86). However, some reports stated that the activity of acid phosphatase was uninfluenced (63,91).

The effect of "inert" IUDs on several biochemical parameters in the uterus or in the endometrium has been studied: the oxygen uptake was increased (26,82), the mucopolysaccharide content was either increased (8,22,23) or decreased (24), the normal increase in nonphospholipid to phospholipid ratio was retarded (49), the protein content was increased (81), the RNA and DNA concentration was unchanged, decreased or increased (72,79,81,82,86,152), the incorporation of different labelled precursors into RNA gave contradictory results (79,90), the glycogen content was either increased or unaltered (8,60,80,83,117,151). Some of these effects might be secondary to inflammatory changes. Thus, Joshi (80) reported an accumulation of glycogen also when the inflammatory response was elicited by human serum albumin. However, an accumulation of glycogen in the endometrium was found in wearers of the Cu-T despite a mild inflammatory reaction (98,110,111,126). Sudha (138), found no increase in the mucopolysaccharide content in the absence of inflammation.

Several authors have reported evidence in favour of an altered uterine sensitivity to oestrogens (13,72,88,137) and progesterone (8,79,90). Joshi (79) found no change in uterine sensitivity to oestrogens and Laumas and Farooq (89) reported normal uptake and disappearance of labelled progesterone in rat uteri in the presence of "inert" IUDs. All except one (137) of these studies were made on uteri without separation of the endometrium from the myometrium.

Obviously, "inert" IUDs have several effects on the endometrium. It is important to consider this, when the mode of action of "active" IUDs is studied, as some of the effects obtained in such studies, might be due to the presence of the "inert" part of the IUDs.

Effect of copper IUDs on the endometrium

In contrast to most "inert" IUDs the presence of copper IUDs resulted in slight or no traumatic effects in women (57,130,158). Salaverry et al. (130) reported "irregular" maturation in 25 out of 132 endometrial samples. Neither Zipper (158) nor Hagenfeldt (57) found any morphological evidence for a disturbed endometrial maturation. In rabbits Zipper (157) noted an intense proliferation of the stromal part of the endometrium on the tenth day of pregnancy. During the first four days of pregnancy in rabbits no lightmicroscopic changes could be found (1), but deciduomata developed on the fifteenth day (145). Similar to "inert" IUDs, copper IUDs inhibit the decidual reaction in rats (17,148).

Slight or no inflammatory reaction is reported in women wearing copper IUDs (57,130,158). In experimental animals (rats, rabbits, monkeys), however, a copper IUD elicits a marked inflammatory response (30,145) even stronger than in the presence of "inert" devices (30).

Contrary to "inert" IUDs, copper IUDs have been found to decrease alkaline phosphatase activity of human endometrium in vivo and in vitro (56,150). In rats, the activity of this enzyme is increased in the presence of "inert" and copper IUDs (20,21). Copper IUDs as well as "inert" IUDs increase the activities of acid phosphatase (1,21,56) and beta-glucuronidase (21). Hagenfeldt (56), however, reported a slight decrease in beta-glucuronidase activity in women wearing the Cu-T and the activity of lactic dehydrogenase was unaltered. This enzyme is increased in copper IUD influenced rat uteri (21). Various other enzyme activities (e.g. catalase, alfa-amylase, glycogen synthetase) are reported to be influenced in the presence of copper IUDs (31,126).

Many biochemical parameters in the endometrium were found to be influenced in the presence of copper IUDs, e.g., the protein concentration was increased (55), the RNA concentration was slightly increased (55) and the incorporation of labelled sulphate into mucoproteins was decreased (124).

The metabolism of glycogen in human endometrium, in the presence of the Cu-T, is disturbed, as glycogen accumulates and its secretion and degradation are deficient (98,110,111,126). An "inert" IUD caused no change in the glycogen content of human endometrium in a histochemical study (60). However, as pointed out above "inert" IUDs cause an accumulation of glycogen in rat uteri (8,80,117,151).

Hagenfeldt found no change in the DNA content of the endometrium, in the presence of the Cu-T when determined in endometrial bio-

psies (56). However, a decreased DNA content in isolated human endometrial cells during the late proliferative phase was described (58). The incorporation of ^{14}C -thymidine into DNA was reduced in oestradiol-treated rats (124). These findings suggest an effect of copper on the DNA synthesis in the endometrium.

It has been suggested that the hormonal responsiveness of the endometrium is changed in the presence of copper IUDs. Johansson (76) showed that the presence of copper depressed the specific binding of oestradiol to uterine cytosol. An in vitro study (50) reported that the uptake of labelled progesterone was higher and the uptake of labelled oestradiol was lower in rat uteri exposed to copper IUDs for 30 days. This resulted in a lower oestrogen/progesterone ratio in the copperinfluenced rat uteri. In in vivo studies the uptake of labelled oestradiol was unaltered in rat uteri during the first days after insertion of the copper devices (2,17) but increased after eight days and four weeks (2,4). The uptake of labelled progesterone was uninfluenced by the presence of copper IUDs in one study (4) and the rate of implantation could not be altered by exogenous administration of oestrogen and progesterone, alone or in combination (18). Thus, there are no conclusive data concerning an altered hormonal responsiveness of the endometrium in the presence of copper IUDs.

The mechanism of action of "inert" and copper IUDs seems to be complex, but endometrial function is disturbed in several ways, which might seriously interfere with normal implantation.

AIM OF THE PRESENT INVESTIGATION

The main objects of this investigation were:

1. To study the effect of copper and "inert" IUDs on the incorporation of ^3H -TdR and 5- ^3H -UR into endometrial cells during early pseudopregnancy and pregnancy in an experimental model in rabbits.
2. To study the effect of copper and "inert" IUDs on some endometrial enzymes and on the morphological development of the endometrium during early pseudopregnancy and pregnancy.
3. To study the effect of cupric ions on ^3H -TdR incorporation into endometrial cells obtained during early pseudopregnancy and to see whether the effect of cupric ions could be modified by the presence of ligands, i.e. amino acids and proteins.

The results are, or will be, published in five papers, which form the basis of this thesis. The papers are referred to in the text by their Roman numerals.

I. "The effect of a copper IUD and 'inert' IUDs on the incorporation of ^3H -thymidine and 5- ^3H -uridine into the endometrium of the rabbit after stimulation with human chorionic gonadotropin" (together with Leif Håkansson).
Acta Obstet. Gynec. Scand., 54:173-81, 1975.

II. "Effect of a copper IUD on the incorporation of ^3H -thymidine and 5- ^3H -uridine into the endometrium of the rabbit during early pregnancy"
J. Reprod. Fert., 42:447-58, 1975.

III. "Effect of cupric ions on the incorporation of ^3H -thymidine and 5- ^3H -uridine into nucleic acids of rabbit endometrial cells. An in vitro study"
(together with Leif Håkansson).
Submitted for publication in *Contraception*, 1975.

IV. "Effect of a copper and a plastic IUD on the histochemistry of endometrial enzymes in the rabbit during early pseudopregnancy" (together with Leif Håkansson and Inga Hägerstrand).
Accepted for publication in *Acta Obstet. Gynec. Scand.*, 1975.

V. "The effect of a copper IUD and various 'inert' IUDs on the histological picture of the rabbit endometrium during early pseudopregnancy and pregnancy"
(together with Leif Håkansson and Inga Hägerstrand).
Accepted for publication in *Acta Obstet. Gynec. Scand.*, 1975.

TECHNICAL ASPECTS AND STATISTICAL CONSIDERATIONS

Rabbits were chosen as experimental animals because the reproductive physiology has been thoroughly studied, because the double uterus permits simultaneous evaluation of the effects of "inert" and copper IUDs within the same animal, and because "inert" IUDs have an antifertility effect which is enhanced by the addition of copper (18,19,38,92,104,122,157). In rabbits, ovulation occurs 12-15 hours after coitus or the injection of human chorionic gonadotropin (HCG), but in the presence of IUDs ovulation occurs somewhat later (7,71,100). The progesterone released previous to ovulation induces a pronounced proliferation, the so-called progestational proliferation (32,93,96). A peak of mitotic activity occurs on days two and three post coitum (p.c.); on the fifth day p.c. there is a very low mitotic activity. The blastocyst implants on day seven. The present investigation studied the first five days of pseudopregnancy or pregnancy.

Pseudopregnancy was induced by an intravenous injection of 75 IU HCG. The hormonal stimulation of the endometrium in pseudopregnancy differs somewhat from that in normal pregnancy (16,39,64,65,134), e.g. the symplastic transformation of the endometrium is premature in pseudopregnancy (e.g. 32) and the composition of the uterine fluid is different (e.g. 87). The incorporation of 5-³H-uridine differed during early pseudopregnancy and normal pregnancy in the present study (see papers I,II). It is extremely laborious to work with pregnant animals (at least when mating must take place at a definite time) the initial study (see paper I) on the effect of IUDs on the DNA and RNA synthesis of the endometrium was carried out in pseudopregnant animals. The effects obtained in this study were then confirmed in pregnant animals (see paper II). Furthermore, no differences between pseudopregnant and pregnant animals were found concerning the effects of IUDs on the histological picture of the endometrium (see paper V).

Four types of IUDs were used in the present study (paper I gives a detailed description of the IUDs). As the mere presence of an "inert" IUD might have effects on the endometrium, the effect of the copper IUD (Cu-IUD) was always compared with that of an "inert" plastic IUD (Pl-IUD_{1.0}) of the same size. This IUD is somewhat stiffer than the Cu-IUD and has a smoother surface, therefore the effect of a platinum IUD (Pt-IUD) was also studied. This IUD, except for the metal, is identical with the Cu-IUD. The core of the Cu-IUD and the Pt-IUD was made of a plastic IUD (Pl-IUD_{0.5}) half the thickness of the Pl-IUD_{1.0}. The IUDs were inserted into the uterus under sterile conditions. This is important in order to permit an evaluation of the inflammatory

response elicited by the IUDs. The animals were allowed to recover for five days before pseudopregnancy or pregnancy was induced. No corpora lutea were found at the insertion of the IUDs, and the present study used only animals that had ovulated after HCG injection or coitus. The length of the IUDs was 50 mm, larger IUDs were found to penetrate the uterine wall too often. In order to avoid differences in the concentration of copper and in mechanical traumatization only the part of the uterus corresponding to the length of the IUD was studied (the so-called IUD-part).

The animals were killed at different times after mating or HCG injection. The internal genitalia were taken out and the uteri were trimmed. The uterine horns were opened by a longitudinal incision along the mesometrial wall and the endometrial surface was gently rinsed with Parker 199 or Locke's (97) solution. Luminal inflammatory cells, and possibly also loosely attached superficial endometrial cells and perhaps especially Cu-IUD influenced endometrial cells, were thus washed away (1). The loss of some Cu-IUD influenced cells as a result of this procedure would, if anything, tend to cause an underestimate of the effect of copper on DNA synthesis obtained in the present study (see papers I,II). The endometrium of the uterine horns corresponding to the IUD-part was then scraped off the myometrium with a sharp curette and put into the incubation medium at 37°C. The endometrial cells were brought into a suspension of small tissue fragments and single cells (for a more detailed description of the experimental procedures see papers I,II,III).

In some experiments the cells were incubated for one hour with either tritiated thymidine (^3H -TdR) or uridine (^3H -UR) and in other experiments the cells were preincubated with cupric ions at various concentrations for three hours and then incubated with these labelled precursors for a further hour. As a measure of incorporation of labelled precursors the following expression, which gives near normally distributed variates (see 112), was used:

$$\underline{a} = 100 \times \log_{10} \frac{\text{cpm} \times 10^4}{\text{AES} \times (\text{DNA})}$$

where AES is the value of the external standard and DNA is the amount of DNA in the test solution expressed as the extinction value (OD₄₉₀) corrected for the standard curve.

The total amount of DNA in the endometrium of the IUD part of the uterus, was determined and expressed in OD₄₉₀ units. As the uterine horns were divided and only part of the endometrium was used, the results might be influenced by differences in the degree of contraction between differently treated horns. However, this is unlikely, as a reduced amount of DNA was found only when the Cu-IUD was compared with the Pl-IUD_{1.0} or the Pt-IUD (see papers I,II) and not when the Cu-IUD was compared with the Pl-IUD_{1.0} in nonstimulated animals or when the Pl-IUD_{1.0} was compared with the other types of IUDs or shamoperation. Furthermore, the amount of DNA in the Cu-IUD influenced endometrium is only 20-50 % of that in the Pl-IUD_{1.0} influenced endometrium. This large difference can hardly be due to inappropriate division of the uterine horns.

The number of inflammatory cells might influence the total amount of DNA, but as shown in the histological study (paper V) there is an increased number of inflammatory cells in the presence of the Cu-IUD, which would tend to increase the total amount of DNA. Moreover, the amount of DNA due to inflammatory cells seems comparatively small as a comparison of the Pl-IUD_{1.0} with the Pt-IUD shows no differences in the total amount of DNA (see Table III, Group V, paper I), although the Pt-IUD elicits an inflammatory response comparable to that found in the presence of the Cu-IUD (see paper V).

⁵-³H-UR was chosen in these studies as an RNA precursor, as the tritium atom is lost when deoxyuridine monophosphate is methylated to thymidine monophosphate and incorporation via deoxycytidine phosphate seems to be of no importance in these cells, as in three experiments, only 3-4 % of the radioactivity of generally labelled uridine is incorporated into DNA (unpublished data). Thus, the radioactivity of ⁵-³H-UR is reasonably incorporated into RNA. It is remarkable that the incorporation of ⁵-³H-UR gradually decreases during the first five days after HCG injection, whereas it increases during the first days after coitus and reaches a peak on days two and three. This difference might be due to differences in the hormonal stimulation of the endometrium, as the luteinizing hormone properties of HCG can elicit an increased oestradiol release. Murdoch and O'Shea (107) reported a decreased incorporation of uridine into RNA of rabbit endometrial cells *in vitro* 5 hours after HCG injection. They (107,133) discussed the influence of the size of the uridine nucleotide pool on the incorporation of labelled uridine and suggested that the decreased incorporation of ⁵-³H-UR after HCG stimulation might be due to an increase in the pool size resulting in dilution of the labelled precursor. The higher incorporation of ⁵-³H-UR on the fifth day of pseudopregnancy and pregnancy in the presence of the Cu-IUD found in the present investigation (see papers I,II) can thus mirror an increased RNA

synthesis or be due to a decreased size of the uridine nucleotide pool. However, it is unlikely that copper interferes with the synthesis of this pool, as copper had no influence on the incorporation of 5-³H-UR in nonstimulated rabbits (see paper I).

Tritiated thymidine (³H-TdR) was incorporated into DNA, as an exclusively nuclear labelling was found in a fairly low number of endometrial cells in autoradiographic preparations. Differences in the incorporation of ³H-TdR into endometrial cells from differently treated horns might be due to differences in degradation of the precursor (25,112). However, in this study possible differences in the degree of thymidine degradation did not seem to influence the differences in ³H-TdR incorporation (see papers I,II), as ³H-TdR incorporation was higher in the Pl-IUD_{1.0} or Pt-IUD influenced horns than in the Cu-IUD influenced horns at 48-72 hours p.c. or after HCG injection, despite more endometrium in the control horns and a similar inflammatory response in the presence of the Cu-IUD and the Pt-IUD. As pointed out above in the discussion of the incorporation of 5-³H-UR changes in the size of nucleotide pools can influence the rate of incorporation of labelled precursors. However, there are several reasons for believing that the inhibitory effect of copper on ³H-TdR incorporation in the present study actually mirrors a decreased DNA synthesis. The ³H-TdR incorporation reached a maximum 48-72 hours after HCG injection or coitus in shamoperated as well as in IUD influenced uteri and then decreased. This agrees well with earlier reports (32, 93,96) on mitotic index and labelling frequency data. Furthermore, the increase in ³H-TdR incorporation during early pseudopregnancy and pregnancy in the present study, was paralleled by an increase in DNA content of the endometrium, and the reduced ³H-TdR incorporation into Cu-IUD influenced endometrial cells agrees with the lesser increase in the amount of DNA and with the inhibited endometrial proliferation in the presence of the Cu-IUD as demonstrated in a histological study (see paper V).

The effects of the various IUDs on ³H-TdR and 5-³H-UR incorporation into endometrial cells were evaluated as follows: the $\bar{a}$ -value for each uterine horn (the mean of double values) was determined and the difference between these values for the two horns of each animal calculated. If two different IUDs influence the incorporation of labelled nucleosides in the same way and to the same extent, the $\bar{a}$ -values will scatter around zero. The deviation from zero was tested in an analysis of variance where all subgroups (different times after mating or HCG injection) in each experimental group were included. If the differences of a group were found to deviate significantly from zero the deviation of the mean differences of each subgroup from zero was analysed by means of a two-sided t-test (for further details

see paper I). These statistical methods were also used to analyse differences in the total amount of DNA between differently treated uterine horns. The total amount of DNA was then expressed in logarithmated OD490 units (see papers I,II).

The effect of cupric ions on ^3H -TdR or $5\text{-}^3\text{H}$ -UR incorporation into endometrial cells in vitro is expressed as the difference between the a-value recorded in control tubes and the a-value recorded at a certain cupric ion concentration (the so-called effect-value). In order to evaluate the statistical significance of these effects two-sided t-tests and Wilcoxon's test for paired differences were used (for further details see paper III).

EFFECT OF IUDS ON THE DNA SYNTHESIS AND THE DNA CONTENT OF THE ENDOMETRIUM

The ^3H -TdR incorporation into endometrial cells increased in shamoperated horns during the first two days of pseudopregnancy. On the fifth day, the incorporation of this precursor had decreased to levels lower than in nonstimulated animals (see Fig. 2 B, paper I). These results as discussed above, agree well with earlier reports. No significant differences in ^3H -TdR incorporation were found between Pl-IUD_{1.0} influenced and shamoperated horns or between Pl-IUD_{1.0} and Pl-IUD_{0.5} influenced horns (Table I Groups III-IV, paper I). Some groups showed fairly large non-significant differences, which might have some biological importance; for example at 0 hours, when the effect of the Pl-IUD_{1.0} was compared with that of the Pl-IUD_{0.5} and shamoperation (Table I Groups III-IV, paper I). The Pt-IUD caused a significantly lower incorporation of ^3H -TdR (at 0 and 120 hours) than did the Pl-IUD_{1.0} (Table I Group V, paper I). The reason for this is obscure.

The total amount of DNA in the endometrium of the IUD-part of the uterine horn increased markedly during early pseudopregnancy and pregnancy in the shamoperated horns and in the presence of "inert" IUDs. No significant differences were found when the effect of the Pl-IUD_{1.0} was compared with that of shamoperation, the Pl-IUD_{0.5}, or the Pt-IUD.

Thus, "inert" IUDs did not influence the DNA content in the present study (see Table III Groups III-V, paper I), whereas both increased and decreased DNA concentrations have been reported in studies in other species under various hormonal conditions (81,82,86,152).

When the effect of the Cu-IUD was compared with that of the Pl-IUD_{1.0} in nonstimulated rabbits, the incorporation of ^3H -TdR was significantly higher in horns containing the Cu-IUD (Table I Group II, paper I). No significant differences were found at 0 and 120 hours, whereas the difference at 48 hours was statistically significant. At present it is obscure why this effect of the Cu-IUD appears only at this time.

On days two and three of pseudopregnancy (Table I Group I, paper I) and pregnancy (Table I Group I, paper II) the incorporation of ^3H -TdR was significantly lower in the Cu-IUD influenced endometrium than in the Pl-IUD_{1.0} influenced endometrium. On the fifth day the incorporation of ^3H -TdR had decreased markedly in the Pl-IUD_{1.0} horn and also in the Cu-IUD horn, although it was significantly higher in the latter (see Table I Group I, paper I and Table I Group I, paper II). Almost identical results were obtained when the effect of the Cu-

IUD was compared with that of the Pt-IUD in early pregnancy (Table 1 Group II, paper II). However, as the table shows the difference between the effects of the Pt-IUD and the Cu-IUD are more pronounced than those between the Pl-IUD_{1.0} and the Cu-IUD. This agrees well with the differences between the effects of the Pl-IUD_{1.0} and the Pt-IUD on ³H-TdR incorporation described above (see Table I Group V, paper I).

The total amount of DNA is increased also in the presence of copper (see Fig. 5, paper I and Figs. 4 and 5, paper II), but to a lesser extent, which agrees well with the reduced ³H-TdR incorporation during the time of maximum proliferative activity and also with the lower labelling frequency of copperinfluenced endometrial cells 72 hours p.c. (see Table 4, paper II).

The effect of cupric ions - in the same concentration range as reported in human copper IUD exposed endometrium (55,62) - on ³H-TdR incorporation into rabbit endometrial cells was studied in in vitro experiments (see paper III). Cupric ions (3-10 µg/ml) were found to significantly inhibit the incorporation of ³H-TdR into endometrial cells, obtained on days two and five of pseudo-pregnancy, whereas 5-³H-UR incorporation was not significantly influenced by cupric ions in the same concentrations. Practically identical results were obtained, when the effect of cupric ions on ³H-TdR incorporation into human endometrial cells (obtained on cycle days 13-16) were studied in the same type of in vitro experiments (see Fig. 1).

The inhibition of the DNA synthesis in copperinfluenced endometrial cells described in the present study agrees with the results obtained by Prager (124) and Hagenfeldt (58). However, it is possible that the reduced incorporation of ¹⁴C-thymidine observed by Prager does not mirror an effect on the DNA synthesis. She administered the labelled nucleoside into the uterine lumen of oestradiol-treated rats fitted with copper IUDs, and as it has been demonstrated that copper elicits a more marked inflammatory response than "inert" IUDs in rats (30), the lower uptake can possibly be due to an increased breakdown of the labelled precursor by polymorphonuclear leukocytes (25) or to an increased resorption from the endometrium owing to the inflammatory reaction. The DNA content in isolated endometrial cells obtained by a brush technique was found to be decreased in women who had used the Cu-T device for six months (58). This effect, however, was not accompanied by a corresponding decrease in the DNA content of the endometrium as judged from DNA determinations in endometrial biopsies (55).

The DNA synthesis, in the present investigation, was inhibited in the presence of the Cu-IUD on days two and three of pseudo-pregnancy and pregnancy whereas it was higher in the Cu-IUD influenced horns on day five (see papers I,II). In in vitro

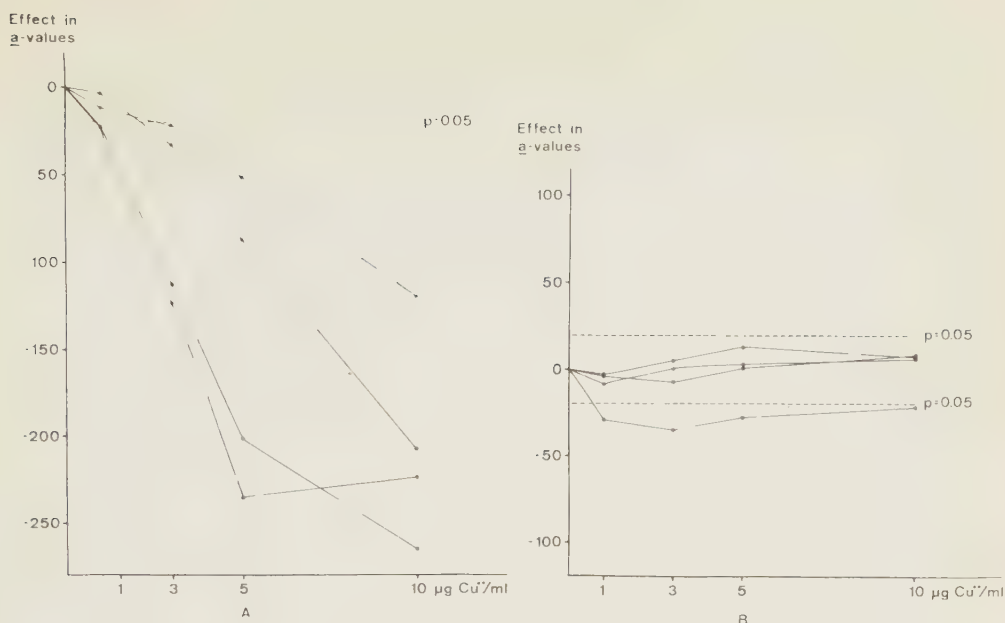


Fig. 1. shows the effects of different cupric ions on the incorporation of ^3H -TdR (A) and $5\text{-}^3\text{H}$ -UR (B) into human endometrial cells obtained during the late proliferative phase (cycle days 13-16). In order to evaluate the statistical significance of these effects, the errors of the effect values, i.e. the difference between mean a-value in control tubes and mean a-values in tubes containing different concentrations of cupric ions, were calculated as follows: The dispersion of the a-variables within identically treated tubes was estimated from all triplicate determinations and was found to be 12.19 based on 61 d.f. Bartlett's test was applied to see whether different endometria or differently treated groups (different cupric ion concentrations) varied with respect to this dispersion. This was not the case, except for two groups. As these had a lower error variance, they were excluded in the calculation of the general error variance. But in order not to overestimate the significance of the effects obtained in these experiments, the general error variance was used to calculate the significance levels. The error of the mean difference between two sets of observations, each based on

three identically performed tests is $12.19 \sqrt{\frac{1}{3} + \frac{1}{3}} = 9.96$.

The 5 per cent significance level is thus a mean difference of 19.92 and is represented by the dotted line. The 1 and 0.1 per cent significance level is 26.49 and 34.46, respectively.

experiments cupric ions inhibited, to the same extent, the incorporation of ^3H -TdR into endometrial cells, obtained on days two and five of pseudopregnancy. Thus, there seem to be factors in vivo, that can modify the effect of copper.

Tobert (145) demonstrated that the solubility of copper was lower in pseudopregnant than in oestrus rabbits, and Wilson (150) found an increased solubility of metallic copper in the human secretory phase, which agrees well with the higher copper concentration found in secretory endometrium by Hagenfeldt (55). Thus, the differences in the effect of the Cu-IUD on the DNA synthesis found on days two and five of pseudopregnancy and pregnancy in the present study, might to some extent be due to changes in the copper concentration on these two occasions. Furthermore, the effect of cupric ions can be modified by the presence of amino acids and proteins. Odeblad and Minc (114) found a reduction of the inhibitory effect of cupric ions on sperm migration in vitro in the presence of threonine and histidine, and the effect of cupric ions on two-cell embryos in vitro was diminished when bovine serum albumin was added to the medium (12). In the present investigation, threonine, histidine and human serum albumin, in in vitro experiments, significantly reduced the inhibitory effect of cupric ions (5 $\mu\text{g}/\text{ml}$) on ^3H -TdR incorporation into endometrial cells obtained during early pseudopregnancy (see paper III). Thus the higher protein concentration in rabbit uterine fluid on the fifth day of pseudopregnancy and pregnancy compared with that on the third day (87), might reduce the copper effect.

EFFECTS OF IUDS ON THE RNA SYNTHESIS OF THE ENDOMETRIUM

No difference in the incorporation of $5\text{-}^3\text{H-UR}$ was found when the effect of the $\text{Pl-IUD}_{1.0}$ was compared with shamoperation in pseudopregnant animals, nor were there any differences in the effects of the $\text{Pl-IUD}_{0.5}$, the $\text{Pl-IUD}_{1.0}$ or the Pt-IUD (see Table II Groups III-V, paper I).

The effect of the Cu-IUD was compared with that of the $\text{Pl-IUD}_{1.0}$ and no difference was found in nonstimulated animals (see Table II Group II, paper I), but it caused a significantly higher incorporation of $5\text{-}^3\text{H-UR}$ on the fifth day of pseudopregnancy and pregnancy and there was a tendency towards a higher incorporation into the copperinfluenced endometrial cells at all times after HCG stimulation or coitus (see Table II Group I, paper I and Table 2 Groups I and II, paper II). When the effect of the Cu-IUD was compared with that of the Pt-IUD , the incorporation of $5\text{-}^3\text{H-UR}$ was found to be significantly higher in the presence of the Cu-IUD also in oestrus animals (see Table 2, Group II, paper II). Thus, again, somewhat different results were obtained when the Pt-IUD was used as a control IUD instead of the $\text{Pl-IUD}_{1.0}$ (cf. the results obtained when the effect of IUDs on $^3\text{H-TdR}$ incorporation was studied).

Laumas and Yadava (90) and Joshi (79) studied the effect of "inert" IUDs on the incorporation of labelled precursors into RNA under different hormonal conditions and obtained contradictory results. The RNA concentration has been reported to be unaltered (82,86) or increased (81,152) in the presence of "inert" IUDs in various species. Thus no consistent effect of "inert" IUDs on RNA synthesis has been described. The slight increase in RNA content (mg/g wet weight) reported by Hagenfeldt (56) in human endometrium in the presence of the Cu-T agrees with the present results.

HISTOCHEMICAL AND HISTOLOGICAL STUDIES ON THE EFFECTS OF IUDS ON THE MATURATION OF THE ENDOMETRIUM

A histochemical study (paper IV) described the activities and distribution of the endometrial enzymes alkaline- and acid phosphatase and beta-glucuronidase. The appearance of the activities of these enzymes, as studied in the present investigation, in shamoperated horns of pseudopregnant animals, agrees well with that described by Petry et al. (120). An "inert" IUD (the Pl-IUD_{1.0}) evoked a slight increase in the activity of the two lysosomal enzymes (i.e. acid phosphatase and beta-glucuronidase) and the Cu-IUD caused a more marked increase in the activities of these enzymes. "Inert" and copper IUDs have generally been found to increase acid phosphatase and beta-glucuronidase activities (1,11,20,21,56,80,81,86), whereas the acid phosphatase activity in one study was unaltered in rabbits (91) and the beta-glucuronidase activity was slightly decreased in one report on women (56). As paper IV pointed out, the increased activities of these enzymes in some of the above cited studies might be due to the inclusion of leukocyte lysosomal enzyme activity in the biochemical determinations.

An "inert" IUD had no effect on the activity of alkaline phosphatase in the present study. Unaltered or increased activity of this enzyme was found in the presence of "inert" IUDs (20,60,63,81,91,132). The epithelial activity of alkaline phosphatase in the copper-influenced endometrium was almost completely abolished (see Fig. 1 B, paper IV). This result agrees well with earlier reports (56,150). Alkaline phosphatase requires the presence of zinc for optimum activity and as discussed by Zipper (158), the activity of this enzyme might therefore be affected by other cations, e.g. cupric ions.

Paper V (Figs. 1-2) describes the histological picture of the endometrium in the presence of the various "inert" IUDs and the Cu-IUD. The appearance of the endometrium in shamoperated horns (of animals fitted with the Pl-IUD_{1.0} in the other horn) agreed well with earlier reports on the development of the endometrium during early pseudopregnancy in rabbits (32). The present study revealed no obvious differences between the appearance of the endometrium in pseudopregnancy and pregnancy (cf.6). All types of IUDs used in this study caused local endometrial erosions sometimes extending to mucosal necroses. These effects were more pronounced in the presence of the Pt-IUD and the Cu-IUD. Several authors have described traumatic effects of IUDs in women (9,14,59,60,63,70,113,127,129,149) and animals (3,15,28,84,91,102). The Cu-IUD and also the Pt-IUD caused a more marked inflammatory response in our rabbits than did the plastic IUDs. This agrees with earlier reports on rodents

and monkeys (30,145). However, the Cu-T device causes only slight or no inflammatory reaction in women (57,130).

The proliferation and the development of rabbit endometrium during early pseudopregnancy and pregnancy were uninfluenced by "inert" IUDs in the present study. However, the Cu-IUD caused a marked inhibition of the proliferation of the endometrium: the glands were fewer in oestrus rabbits, on the second and third days after HCG injection or coitus the surface epithelium was less pseudostratified, showed less minor folding and fewer mitotic figures, and on the fifth day there was a reduced branching of minor folds with fewer interconnecting bridges and fewer glands (see Figs. 1-2, paper V). Furthermore the response of the endometrium, in castrated rabbits, to exogenously administered 17-beta-oestradiol was very poor (see Fig. 3, paper V). Inhibited growth of human cells in culture, in the presence of a copper IUD and cupric ions, was described by Jones (77) and Hounsell (66). These results are in good agreement with the present findings. Some authors reported delayed development of the endometrium (5,9,129,149); others found a normal (60,70,139) or premature (153,154,155) development. Thus there is no general agreement on the morphological changes of the endometrium in the presence of IUDs.

GENERAL SUMMARY AND CONCLUSIONS

The present thesis studied the effect of a copper IUD and various "inert" IUDs on the endometrium during the first five days of pseudopregnancy and pregnancy in rabbits.

Compared with shamoperation two plastic IUDs had no influence on the ^3H -TdR incorporation into endometrial cells. But a platinum IUD caused a lower ^3H -TdR incorporation than did one plastic IUD. However, compared with the effect of shamoperation none of the "inert" IUDs influenced the total amount of DNA in the endometrium.

The copper IUD, whose effect was always compared with that of an "inert" IUD, caused a higher incorporation of ^3H -TdR in nonstimulated rabbits. On the second and third day of pseudopregnancy or pregnancy the copper IUD significantly reduced ^3H -TdR incorporation into endometrial cells. The labelling frequency of epithelial endometrial cells obtained on the third day of pregnancy was lower in copperinfluenced endometrial cells. This inhibition of the DNA synthesis, which is considered to be an effect of copper, resulted in a lesser increase in the total amount of DNA in the copperinfluenced endometrium.

Paper III demonstrated that cupric ions, in the same concentration range as found in human endometrium in wearers of copper IUDs, significantly inhibited ^3H -TdR incorporation into endometrial cells obtained on the second and fifth day of pseudopregnancy. This study also showed that the inhibitory effect of cupric ions on ^3H -TdR incorporation could be reduced by the addition of threonine, histidine and human serum albumin to the incubation medium. ^3H -TdR incorporation into human endometrial cells (obtained during the late proliferative phase) was also significantly inhibited by cupric ions. $5\text{-}^3\text{H}$ -UR incorporation was not significantly influenced by cupric ions, either in rabbit or in human endometrial cells in these in vitro experiments.

"Inert" IUDs did not significantly influence $5\text{-}^3\text{H}$ -UR incorporation during early pseudopregnancy, nor did the copper IUD alter $5\text{-}^3\text{H}$ -UR incorporation into endometrial cells of nonstimulated animals, but it caused a higher incorporation of this labelled precursor on the fifth day of pseudopregnancy and pregnancy.

A histochemical study demonstrated that both "inert" and copper IUDs increased the activities of lysosomal enzymes (acid phosphatase and beta-glucuronidase). The activity of alkaline phosphatase in surface epithelial cells of the endometrium was almost completely abolished in the presence of the copper IUD.

Finally, the histological study demonstrated a marked inhibition of the endometrial proliferation during early pseudopregnancy and pregnancy in the presence of the copper IUD. This morphological finding agrees well with the effects of the copper IUD on the incorporation of ^3H -TdR and on the total amount of DNA in the endometrium. Furthermore, this study found a very poor response to 17-beta-oestradiol in castrated rabbits exposed to the copper IUD.

The inhibitory effect of copper on the DNA synthesis and the proliferation of the endometrium during early pseudopregnancy and pregnancy might possibly be due to a nonspecific "toxic" effect. However, this is unlikely, as 5- ^3H -UR incorporation was uninfluenced or, in some experiments, even increased in the presence of copper (see papers I,II,III).

The hormonal initiation of the proliferation during the first hours after mating or HCG injection might be disturbed and copper might also have an inhibitory effect after the initiation of the proliferation. As discussed above, the proliferation is elicited by the action of progesterone on the estrogen-primed endometrium (cf. 27). There are several reasons for believing that the responsiveness of the endometrium to oestradiol is decreased in the presence of copper. A very poor response to this hormone was obtained in castrated rabbits in the presence of the Cu-IUD (Fig. 3, paper V) and the number of glands in oestrus rabbits was reduced after exposure to this IUD for five days (see Fig. 2 A and B, paper V). An adequate lysosomal function seems to be necessary for a normal response to oestradiol in target cells (140,141,142). However, copper accumulates in lysosomes (1,95,147) and the activity of lysosomal enzymes is influenced by the presence of copper IUDs (see paper IV). Thus the oestradiol "priming" of the endometrium in the presence of copper is probably defective and the initiation of the proliferation might be disturbed. However, paper III showed that cupric ions, in in vitro experiments, significantly inhibited ^3H -TdR incorporation into endometrial cells obtained on the second and fifth day of pseudopregnancy. Practically identical results were also obtained when the effect of cupric ions on the incorporation of ^3H -TdR into human endometrial cells was studied in in vitro experiments. Thus copper also has an effect on the proliferation after its initiation.

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